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A new species of *Bipolaris* from the halophyte *Sesuvium portulacastrum* in Guangdong Province, China

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Abstract — A new non-graminicolous species found to infect *Sesuvium portulacastrum* (*Aizoaceae*) in China is introduced. *Bipolaris sesuvii* is characterized by straight, subcylindrical conidia with monopolar germination. Phylogenetic analysis based on ITS rDNA sequences show that *B. sesuvii* clusters with *B. indica*, which is distinguished by its straight, short clavate conidia. The combination of DNA sequence and morphological data indicate that *B. sesuvii* is a distinct species of *Bipolaris*. Pathogenicity tests also confirm that *Sesuvium portulacastrum* (sea purslane) is a natural host of *B. sesuvii* causing leaf lesions, leaf blight, or leaf rot and stem lesions.

Key words — fungus, taxonomy, systematics, phylogeny

Introduction

Among the 115 species of the genus *Bipolaris* listed in Index Fungorum, there are more than sixty non-graminicolous species, including a few species of human pathogens. The graminicolous species of *Bipolaris* were monographed by Sivanesan (1987). Since then, eleven new species of *Bipolaris* (Chiang et al. 1989, Peng & Liu 1989, Sisterna 1989, Alcorn 1990, Chen et al. 2000, Deng & Zhang 2002) have been described.

Bipolaris species are associated with *Cochliobolus* teleomorphs. The similar anamorph genera *Drechslera* and *Exserohilum* are associated with *Pyrenophora* and *Setosphaeria* teleomorphs, respectively. Although Shoemaker (1959) previously included all three anamorph genera within the genus *Helminthosporium* and differences in conidial morphology are sometimes too slight to distinguish the three anamorphic genera (Subramanian & Jain 1966, Ellis 1971, Chidambaram et al. 1973), ascospore shape, septation, and color easily distinguishes *Cochliobolus* from *Pyrenophora* and *Setosphaeria*. For, Where the teleomorph is unknown, many species of *Helminthosporium* sens. lat. have been assigned to any of the three teleomorphic genera, *Drechslera*, *Bipolaris*, or *Exserohilum* (Zhang & Berbee 2001).

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Molecular analyses of various gene regions (e.g. glyceraldehyde-3-phosphate, internal transcribed spacer (ITS), LSU rDNA) have been used to resolve phylogenetic relationships of pleosporaceous fungi (Berbee et al. 1999, Olivier et al. 2000, Zhang & Berbee 2001, Dela Paz et al. 2006, Kodsueb et al. 2006, Zhang et al. 2008).

In *Cochliobolus*, sequence comparisons indicate that the genus is monophyletic and clusters into two groups. Among the many previously misidentified isolates, CBS403.72 has been revised from *Pyrenophora bromi* to *Bipolaris portulacae* (Zhang & Berbee 2001). Some diagnostic characters, such as bipolar germination of conidia, are affected by environmental conditions (e.g. culture media). Bipolar conidial germination is considered an important taxonomic character in *Bipolaris,* but *B. oryzae* conidia regularly exhibit the intercalary germination more commonly observed in *Drechslera.* ITS sequence analyses by Dela Paz et al. (2006), however, identified all isolates as *B. oryzae*, irrespective of whether conidial germination in the culture was observed as bipolar, intercalary, or monopolar. This demonstrates that rDNA sequences (particularly the internal transcribed spacers ITS1 and ITS2) are useful tools for resolving taxonomic relationships within *Bipolaris* and among species of many other genera (e.g. *Ampelomyces,* Liang et al. 2007; *Dactylella,* Chen et al. 2007; *Cordyceps,* Wang et al. 2008; *Xylariaceae*, Peláez et al. 2008).

Recently, in the Zanjiang District of Guangdong Province, China, we encountered a species of *Bipolaris* infecting *Sesuvium portulacastrum* in sand dunes, salt marshes, and mangrove swamps that did not match any currently accepted species of this genus. Here we describe the new species and compare it both morphologically and molecularly (based on ITS sequence data) to other *Bipolaris* species. Inoculation tests were conducted to confirm the pathogenicity of the new *Bipolaris* to *Sesuvium portulacastrum*.

Materials and methods

Collection of isolates

Disease symptoms were noted on leaves and stems of *Sesuvium portulacastrum* (*Aizoaceae*). Isolates of a *Bipolaris* were derived from representative single lesions. Conidia (removed directly from sporulating lesions on leaves) and small pieces of diseased tissue (excised from the lesion margins) were placed on PDA (potato-dextrose agar) containing rifampicin. Isolates (Bp-zj 01, Bp-zj 02, Bp-zj 03) were transferred to fresh PDA plates, and conidia from these cultures were stored in 20% glycerol in 1.5 mL cryotubes at –70 °C.

Morphological and cultural studies

Conidia and conidiophores taken from lesions were examined in distilled water. In order to observe fungal morphology on media, small pieces of frozen conidial suspensions were removed from stock-culture cryotubes without thawing, and

transferred to Petri dishes containing PDA. Single conidial isolates were prepared and grown on PCA (potato carrot agar) and WSA (water agar + wheat straw) from conidia produced on these Petri dishes. Colonies were grown at 25°C under 12 h of alternate darkness and fluorescent light. Measurement and microphotographs were taken from slide mounts in lactophenol.

DNA extraction

Fungal isolates were cultured on PDA at 25°C under cool fluorescent illumination. After four days, five mycelial disks were excised from the margins of colonies and inoculated into 100 mL of liquid growth media (PD broth) in 250 mL flasks, shaken at 200 rpm at 25°C for 3–5 d. Mycelia were harvested by filtration, freeze dried, ground to a fine powder in liquid nitrogen, and then stored at –70°C. About 50 mg of mycelial powder was removed into a sterile 1.5 mL tube, rehydrated in 600 µL of 2 × CTAB buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 30 mM EDTA, 2% hexadecyltri-methylammonium bromide) and incubated in a water bath at 65°C for 30–60 min. Following a phenol/chloroform extraction, the genomic DNA was precipitated by isopropanol in the presence of sodium acetate and visualized in 1% agarose gels after ethidium bromide staining.

Amplification of ITS regions

The rDNA ITS regions were amplified using primers ITS6 (5' GAAGGTGAA GTCGTAACAAGG 3') and ITS4 (5'- TCCTCCGCTTATTGATATGC) (Cooke & Duncan 1997). PCR amplifications were performed in a total volume of 50 mL containing 40 mM of Tris-HCI (pH 8.4), 100 mM of KCI, 3 mM of MgCL2, 400 µM of each dNTP, 1µM of each primer, and 0.5 U of Taq. PCR amplifications were carried out on a DNA thermal cycler (PTC-150 MiniCycler, MJ RESEARCH Corp.). Following an initial denaturation at 95°C for 4 min, the DNA templates were amplified for 35 cycles. Each cycle consisted of a denaturation step at 95°C for 1min, an annealing step at 55°C for 1min, and an extension step at 72°C for 1.5 min. At the end a final extension step (72°C for 10 min) was included. After 4 µL aliquots of the amplification products were electrophoresed on 1% agarose gels, the PCR products were stained with ethidium bromide; successful products produced a single DNA band (corresponding to ~600bp). PCR products were purified using a Biolight PCR Purification Kit (Shanghai Biolight Technology Co., Ltd) according to the manufacturer's instructions.

Sequencing and analysis of rDNA-ITS region

The purified PCR products were submitted to Hangzhou Genomics Institute for sequencing in both directions. Sequence files were assembled and edited, and consensus sequences were constructed using DNAMAN 4.0 (Lynnon bioSoft). The ITS sequences were submitted to the GenBank database, and ITS1, ITS2 and 5·8S rDNA sequences from 33 other fungal isolates were downloaded from GenBank (Table 1); *Leptosphaeria tompkinsii* was selected as an outgroup. Sequences were aligned using CLUSTAL X (Thompson et al. 1994). Phylogenetic analyses were performed using PAUP test Version 4.0b10 (PPC; David Swofford, Smithsonian Institution, Washington DC.). Phylogenetic trees were inferred from the ITS sequence data set using parsimony analysis with all characters weighted equally and unordered.

Pathogenicity testing

Laboratory tests were conducted on apparently healthy leaves collected from the upper part of a plant without any disease occurrence. An aqueous inoculation suspension ($1–2 \times 10^5$ conidia/mL) was prepared from 8–10-day old cultures. Leaves were washed three times with sterile water; each was inoculated with 100 μL of conidial suspension and placed on Petri dishes containing wet filter paper. Control leaves were inoculated with 100 μL sterile water. Petri dishes were placed in a 26–27°C incubator with 12 h of alternate darkness and fluorescent light. The treatments were replicated ten times. The fungus was re-isolated by cutting small portions from the margin of lesions; these were surface sterilized and placed on PDA plates.

Results and discussion

Symptoms on sea purslane

The leaves and stems of *Sesuvium portulacastrum* were infected with leaf lesions, leaf blight or leaf rot, and stem lesions. On leaves, symptoms first appeared as tiny, sunken, light tan to straw-colored flecks with brown borders (Fig. 1). These flecks expanded and became circular or oval brown lesions with a dark brown center. Under moist conditions, the lesions expanded rapidly and the entire leaf or parts of the leaf became water-soaked and brownish. Subsequently, the infected portions became dark purple-brown to dark brown, sometimes with a superficial layer of white mycelium (Fig. 2), and leaf rot or leaf blight occurred as a rapid collapse and drying of the leaves (Fig. 3). Symptoms sometimes occurred on stems as small spots. These spots were light tan to straw-colored, oval to elliptical, with brown borders, often surrounded by a purple-red halo.

New species

Bipolaris sesuvii Jing.Z. Zhang, **sp. nov**. Figs. 3–6

MycoBank MB511136

Conidiophora singularia vel fasciculate, simplicia, raro ramose, medio olivaceobrunnea, versus apicem pallidiora, geniculata vel infra recta, super geniculata, cicatrices, multiseptata, 86–160(–212) μm longa, ad basim tumida 7–10 μm diam, prope basim 6.2–7(–7.5) μm diam, ad apicem 5.5–7 μm diam. Conidia olivaceobrunneae vel brunneae, cylindrical vel late fusoidea, recta, laevia, concolorata, 5–9 distoseptata, 52–77 × 13–16 μm, truncate hilo leviter protrudenti vel non protrudenti..

Type: **CHINA**: Zanjiang, Guangdong, on *Sesuvium portulacastrum* (*Aizoaceae*), 20 Aug. 2006, J.Z. Zhang (**holotype** HMAS 163207).

Etymology: referring to the genus *Sesuvium* on which this fungus was collected and to which it was virulent.

Conidiophores single or fasciculate in small groups, simple, rarely branched once apically, medium olivaceous-brown below, paler at the apex when found on a natural host, geniculate or straight in sterile part, then becoming slightly to distinctly geniculate, cicatrized with scars often inflated and lightly

Fig. 1–3. Symptoms on *Sesuvium portulacastrum* caused by *Bipolaris sesuvii*. 1. Leaf spots. 2 Leaf rot. 3. Leaf blight. Fig. 4–6. Conidia and conidiophore from host. 4. Conidia. 5. Representative conidia. 6. Conidiophore. Bars = 10 μm.

verruculose, multiseptate, 86–160(–212) μm long, swollen to 7–10 μm diam at base, then narrowing to 6.2–7(–7.5) μm diam (middle) and 5.5–7 μm diam (apex). Conidia olivaceous-brown to brown, basal cell concolorous or slightly pale, subcylindrical to broadly fusoid and rounded at ends, straight, smooth, 5–9 distoseptate, 52–77 × 13–16 μm, hilum ≤ 2 μm diam, sometimes slightly protruding.

Cultural characteristics: Colony velvety, floccose, olivaceous-brown to dark brown on PDA. Germination of conidia is monopolar. Conidia maturing with first septum occurring at the median, the second delimiting the basal cell, and the third distal.

Collections examined: **CHINA**: Zanjiang, Guangdong, on *Sesuvium portulacastrum* (*Aizoaceae*), 20 Aug. 2006, J.Z. Zhang (HMAS 163207, holotype); extype living cultures CGMCC 3.9578 (Bp-zj 01), CGMCC 3.9579 (Bp-zJ 02) and CGMCC 3.9580 (Bp-zj 03) deposited in the collection of Biotechnology Institute, Zhejiang University, Zhejiang Province, China.

Table 1. Source isolates and sequences.

Species	Isolate/strain	Source	GenBank
Bipolaris australiensis (M.B. Ellis) Tsuda & Ueyama	Alcorn 8320b	Berbee [a]	AF081450
B. australis Alcorn	Turgeon 77139	Berbee [b]	AF081448
B. cynodontis (Marignoni) Shoemaker	BRIP16821	Goh & Hyde [b]	AF163093
B. dactyloctenii Alcorn	Alcorn 7938-10	Berbee et al. [a]	AF158106
B. eleusines Alcorn & R.G. Shivas	Alcorn 8749c	Berbee [a]	AF08145
B. ellisii (Danquah) Alcorn	Alcorn 81154-1	Berbee et al. [a]	AF071323
B. hawaiiensis (M.B. Ellis) J.Y. Uchida & Aragaki	Alcorn 7612(b)-6	Berbee et al. [a]	AF071324
B. heveae (Petch) Arx	Cyn-2	Tsukiboshi et al. [c]	AB179835
B. indica J.N. Rai et al.	BRIP 17439	Berbee [a]	AF081449
B. kusanoi (Y. Nisik.) Shoemaker	Tsuda Ck2	Yun et al. [a]	AF071352
B. perotidis Alcorn	Alcorn 7846-2	Berbee et al. [a]	AF071320
B. portulacae (Rader) Alcorn	CBS 403.72	Zhang & Berbee [a]	AY004779
B. portulacae	CBS 239.48	Zhang & Berbee [a]	AY004778
B. portulacae	DAOM 208494	Zhang & Berbee [a]	AY004780
B. ravenelii (M.A. Curtis) Shoemaker	Alcorn 7979-6	Berbee et al. [a]	AF071321
B. sesuvii	Bp-zj 01	This study	EF175940
B. sesuvii	Bp-zj 02	This study	EF175941
B. sesuvii	Bp-zj 03	This study	EF175942
B. sorokiniana (Sacc.) Shoemaker	Tinline A20	Berbee et al. [a]	AF071329
B. tetramera (McKinney) Shoemaker	CBS 371.72	Zhang & Berbee [a]	AY004777
B. victoriae (F. Meehan & H.C. Murphy) Shoemaker	Macko HVW	Berbee et al. [a]	AF158109
B. zeae Sivan.	Alcorn 8641a	Berbee [a]	AF081452
Curvularia affinis Boedijn	DAOM 46365	Berbee et al. [a]	AF071335
C. clavata B.L. Jain	DAOM 148084	Berbee et al. [a]	AF071336
C. cymbopogonis (C.W. Dodge) J.W. Groves & Skolko	Alcorn 88109-1	Yun et al. [a]	AF071351
C. gladioli Boerema & Hamers	DAOM 164725	Berbee et al. [a]	AF071337
C. inaequalis (Shear) Boedijn	CBS 185.47	Olivier et al. [d]	AF120261
C. inaequalis	BRIP14448	Goh & Hyde [b]	AF163081 [f]
C. intermedia Boedijn	Alcorn 8797-1	Berbee et al. [a]	AF071327
Drechslera biseptata (Sacc. & Roum.) M.J. Richardson & E.M. Fraser	CBS 108940	Zhang & Berbee [a]	AY004788
D. tritici-repentis (Died.) Shoemaker	DAOM 208990	Berbee et al. [a]	AF071348
D. tuberosa (G.F. Atk.) Shoemaker	DAOM 169286	Berbee et al. [a]	AF071347
Exserohilum gedarefense (El Shafie) Alcorn	8307	Goh & Hyde [b]	AF163068
E. monoceras (Drechsler) K.J. Leonard & Suggs	DAOM 208988	Berbee et al. [a]	AF071340
E. rostratum (Drechsler) K.J. Leonard & Suggs	BRIP23191	Goh & Hyde [b]	AF163066
Leptosphaeria tompkinsii El-Ani	IP 1156.77	Desnos et al. [e]	DQ836789

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[d] Plant Pathology, Cornell University, 334 Plant Science Building, Ithaca, NY 14853, USA
[e] Centre National de Reference Mycologie et Antifongiques, Unite de Mycologie Moleculaire, CNRS FRE2849, Institut Pasteur, 25 rue du Dr. Roux, Paris 75015, France

Comments: Its conidial morphology clearly establishes our specimen as a species of *Bipolaris*. It is morphologically similar to *B. cynodontis* and *B. perotidis* (Sivanesan 1987). The conidia of *B. cynodontis* are of a similar size (37–75 × 10–16 mm) but the conidia are mostly slightly curved and are 3–9, commonly 7–8 distoseptate, with bipolar germination (Sivanesan 1987). The conidia of *B. perotidis* are straight and similar in shape to those of *B. sesuvii*. However, *B. perotidis* has narrower conidia (55–75 × 7.5–12.5 mm) with fewer distosepta (3–7).

Phylogenetic analysis

The ITS1-5·8S-ITS2 rDNA sequences of the three *Bipolaris sesuvii* strains were identical. A GenBank blast search showed a 99% identity similarity with the partial ITS1 of *B. indica* (accession no. AF081449), differing in one base pair in each of the ITS1 and ITS2 regions and a 33 bp indel within ITS1. The *B. sesuvii* sequence also showed a 94% similarity (26 bp difference) with *B. portulacae* (accession no. AY004780) (Fig. 7). Both *B. indica* and *B. portulacae* are considered non-graminicolous species.

A parsimony tree was constructed from the ITS1-ITS2 rDNA regions (424 characters) from 36 fungal isolates (Table 1). The parsimony bootstrap consensus tree (Fig. 8) indicates that *Pyrenophora*, *Cochliobolus* and *Setosphaeria* are monophyletic. The *Drechslera*/*Pyrenophora*, *Curvularia*/*Bipolaris*/*Cochliobolus,* and *Exserohilum*/*Setosphaeria* clades were supported by 100%, 76%, and 99% bootstrap values, respectively.

Bipolaris species clustered into two subclades within the *Curvularia*/*Bipolaris*/*Cochliobolus* clade with only *Bipolaris* isolates grouping in the *Cochliobolus* Group I subclade and *Bipolaris* and *Curvularia* isolates intermingling in the *Cochliobolus* Group II subclade. The three *B. sesuvii* isolates from *Sesuvium portulacastrum* had identical ITS sequences and clustered with *B. indica* in the *Bipolaris*/*Cochliobolus* Group II subclade with a 98% bootstrap value. Sequence variation between *B. sesuvii* and *B. indica* isolates reached a critical intraspecific–interspecific variability value similar to that found for other species, such as *B. portulacae* (Fig. 8). Other *Bipolaris* spp. have shown similarly high levels of intraspecific variability in rDNA regions. For example, the ITS similarity range for *B. portulacae* (CBS 403.72, CBS 239.48, DAOM 208494) is 93–100%. A similar clustering was obtained using neighbour-joining analysis. The three *B. sesuvii* isolates formed a single group together with the *B. indica* isolates (88% bootstrap value; data not shown). The rDNA sequence similarity alone does not provide sufficient information to delimit the relationship between *B. sesuvii* and *B. indica* and *B. portulacae*; however, conidial morphology of *B. sesuvii* differs from *B. indica* and *B. portulacae* (Rai et al. 1969, Alcorn 1990): *B. indica* conidia are clavate, but shorter and wider (17–35 mm) than those of

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Bp-zj 01      CACAAAAAGTATGAAGGCTGCACGCGGCTG------------------------------
DAOM 208494   ******G*A****T****************-----------------------------
BRIP 17439    ********************************TGCCCTCTTGGGGGCCAGCGGCGGGAGGCT

Bp-zj 01      -GATTATCTTTTTCACCCATGTCTTTTGCGCACTTGTTGTTTCCTGGGCGGGTTCGCCCG
DAOM 208494   *A*A****-****C**************************----*********************
BRIP 17439    G******-C*****************************************************

Bp-zj 01      CCACCAGGACCACACAATAAACCTTTTTTTATGCAGTTGCAATCAGCGTCAGTAAAACAA
DAOM 208494   ***************C*************-************************C***T*
BRIP 17439    ************************************************************

Bp-zj 01      ATGTAAA-TCATTTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAAC
DAOM 208494   *******A************************************************************
BRIP 17439    ************************************************************

Bp-zj 01      GCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
DAOM 208494   ************************************************************
BRIP 17439    ************************************************************

Bp-zj 01      CGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCT
DAOM 208494   ************************************************************
BRIP 17439    ************************************************************

Bp-zj 01      CAAGCTTTGCTTGGTGTTGGGCGTTTTTTTGTCTTGCTGCAAGCAAGACTCGCCTTAAAA
DAOM 208494   *************************G**--*******GATCC*********************
BRIP 17439    ************************************************************

Bp-zj 01      CGATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACATTTTTTGCGCTTGCAACCAGC
DAOM 208494   ****************************************A*********************
BRIP 17439    ************************************************************

Bp-zj 01      AAAAGAGGTTGGCGATCCAGCAAGTCCATTTTCTCACTTT
DAOM 208494   ***************************A***C**********
BRIP 17439    ********************************C**********
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Fig. 7. Aligned sequences of the internal transcribed spacer (ITS)1, 5.8S RNA gene and ITS2 of the three *Bipolaris sesuvii* isolates used in this study (Bp-zj 01) together with reference isolates of *B. portulacae* (DAOM 208494) and *B. indica* (BRIP 17439). An asterisk indicates identity with the first sequence and a dash indicates an introduced gap. The alignment was generated using CLUSTAL W (Thompson et al. 1994).

B. sesuvii (Sivanesan 1987); *B. portulacae* conidia are cylindrical, longer (138-190 × 11-14 mm), and with more distosepta (8–11) (Alcorn 1991).

Combining both morphological characteristics and rDNA sequences similarity, we suggest that *B. sesuvii* is an unreported species that differs from *B. indica* and *B. portulacae* as well as other known *Bipolaris* species.

Pathogenicity testing

After 5–6 days *Bipolaris sesuvii* induced small flecks or expanded lesions on all *Sesuvium portulacastrum* leaves tested. Symptoms were similar to those observed in the wild, and no disease was found in the control leaves. In moist

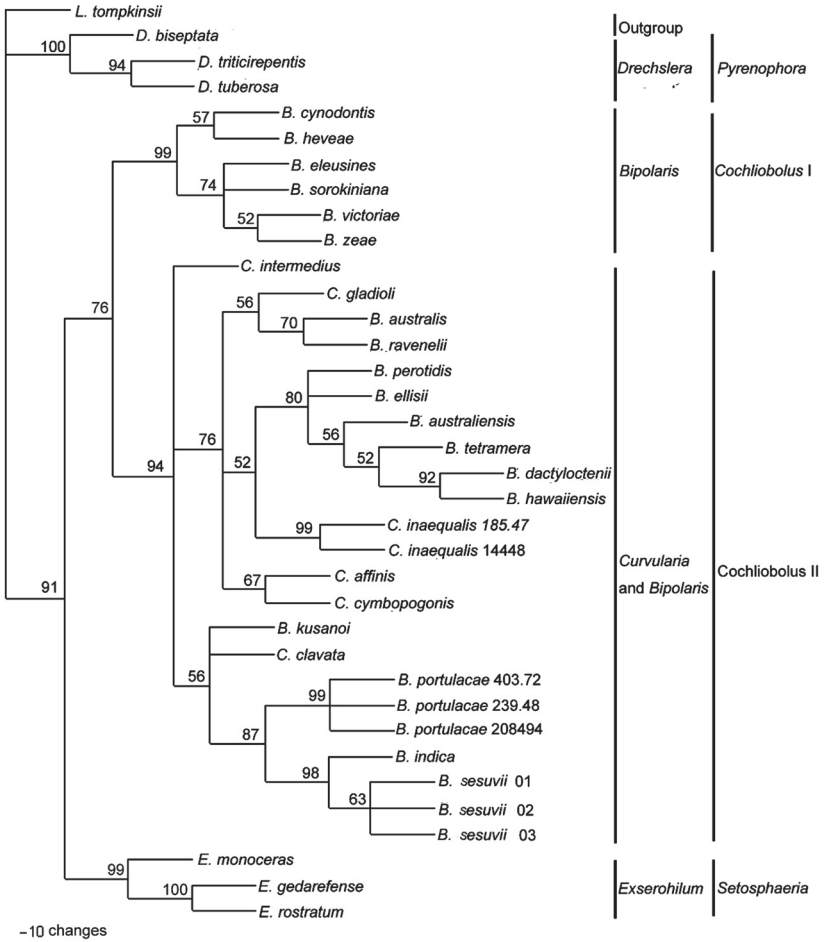

Fig. 8. A parsimony bootstrap consensus tree from complete sequence of ITS1 and ITS2 of 36 ribosomal RNA gene sequences with *Leptosphaeria tompkinsii* as the outgroup. The numbers are the percentage of times groups appeared in 1000 bootstrap replicates.

Petri dishes, lesions on *S. portulacastrum* began to sporulate within 48 h and sporulation was observed on all inoculated leaves by 72 h. The fungus was re-isolated from the infected parts; the colonies and morphology were consistent with original isolates. The results of pathogenicity tests suggest that sea purslane is a natural host of *B. sesuvii*.

The conidial morphological traits of *B. sesuvii* are relatively stable. It produced straight, subcylindrical conidia on different substrates, but there was some variation in conidial septation, length, and diameter. Conidial

Table 2. Conidial dimensions of *Bipolaris sesuvii*.

Substrate	No. of septa [a]	Length (mm)	Width (mm)
Host	6.8 ± 1[b] (5-9)	61.5 ± 7 (55-70)	14.5 ± 1.5 (13-16)
WSA	5.5 ± 1 (4-8)	51 ± 5.6 (42-59)	14.5 ± 1.7 (13-16)
PCA	6.0 ± 1 (4-9)	56.7 ± 14 (40-78)	14.2 ± 1.6 (13-16)
PDA	7.5 ± 1.4 (5-10)	70 ± 10.5 (49-86)	16.0 ± 0.97 (14.5-17.5)

[a] Conidia produced in 8-10-day-old cultures.
[b] Mean and standard deviation from 100 measurements; figures in parentheses represent the range.

dimensions (especially diameter) on WSA (Alcorn 1991) and PCA were closer to those on the host than on PDA (Table 2). The conidial morphology clearly differs from that of other known *Bipolaris* species. Although the teleomorph was not observed in cultures or on the natural host, the *B. sesuvii* teleomorph relationships can be compared by using differences or similarity in conserved DNA sequences to provide a more reliable classification system at the generic and species levels (Shenoy et al. 2007).

Berbee et al. (1999) used the ITS sequences and a portion of the glyceraldehyde-3-phosphate dehydrogenase sequences to evaluate *Cochliobolus* (anamorphs *Bipolaris*) and proposed that *Bipolaris* be divided further, separating species with large, canoe-shaped, gently curving conidia (*Cochliobolus* Group I) from those with short, either straight or curved conidia lacking a gentle curve along the whole spore length (*Cochliobolus* Group II), which are intermixed with *Curvularia* species. All *B. sesuvii* isolates used in this study clustered in the *Cochliobolus* Group II subclade, which contains *Bipolaris* species with short, straight or curved conidia; some species were the same as those analysed by Berbee et al. (1999), Dela Paz et al. (2006), Olivier et al. (2000), and Zhang & Berbee (2001). Although the conidial morphology of *B. sesuvii* is similar to *B. cynodontis* and *B. perotidis*, the phylogenetic analysis did not show a close relationship. The conidial morphology of *B. sesuvii* was distinct from that of *B. indica* and *B. portulacae*, but the phylogenetic evidence indicated that the *B. sesuvii* isolates were related to *B. indica* and *B. portulacae*. The combined molecular-morphological analysis support *B. sesuvii* as an unreported new species.

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